Application No. 09/912,670
Filed: July 23, 2001
TC Art Unit: 1644
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AMENDMENT TO THE CLAIMS

1. (Withdrawn) A method for generating antigen-specific, regulatory CD4+/CD25+ T cells that produce Transforming Growth Factor β (TGF-β), comprising:

exposing CD3-enriched, primed T cells to a specific antigen in the presence of antigen-presenting cells and a composition comprising an effective amount of alpha-Melanocyte Stimulating Hormone (α-MSH) or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof, wherein the specific antigen is an antigen recognized by the primed T cells.

2. (Withdrawn) A method for generating antigen-specific, regulatory CD4+/CD25+ T cells that produce Transforming Growth Factor β (TGF-β), comprising:

exposing CD3-enriched, primed T cells to a T cell receptor (TCR)-crosslinking agent in the presence of an effective amount of α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof.

3. (Withdrawn) The method of claim 1 or 2, further comprising, approximately 4-6 hours after said first exposure step has begun, additionally exposing the primed T cells to an effective amount of Transforming Growth Factor-β2 (TGF-β2).

4. (Withdrawn) The method of claim 3, wherein the exposure to TGF-β2 is achieved by including in the composition, an effective amount of TGF-β2 in an timed-release delivery vehicle.

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5. (Withdrawn) The method of claim 1 or 2, wherein the exposing step is performed *in vitro* under T cell culture conditions.

6. (Withdrawn) The method of claim 1, wherein the exposing step is performed *in vivo* in an animal.

7. (Withdrawn) A method for down-regulating an autoimmune response or other T cell-mediated inflammatory response, comprising:

(a) harvesting T cells from the animal;

(b) inducing TGF-β-producing, regulatory T cells by exposing the harvested T cells *in vitro* to a specific antigen under culture conditions enabling stimulation of at least one primed memory T cell that specifically recognizes said antigen;

(c) exposing the primed T cells *in vitro* to a specific antigen in the presence of a composition comprising an effective amount of alpha-Melanocyte Stimulating Hormone (α-MSH) or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof, and in the presence of at least one T cell receptor(TCR)-crosslinking agent, under T cell culture conditions; and

(d) injecting into an animal, primed T cells treated in accordance with step (c).

8. (Withdrawn) The method of claim 7, wherein step (c) further comprises the addition of an effective amount of TGF-β2, approximately 4-6 hours after the start of the exposure of the primed T cells to the specific antigen and the α-MSH.

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9. (Withdrawn) The method of claim 7 or 8, wherein, between steps (c) and (d), the primed T cells treated in accordance with step (c) are enriched for CD4+/CD25+, TGF-β-producing T cells.

10. (Withdrawn) The method of claim 7 or 8, wherein the TCR-crosslinking agent is an anti-CD3 monoclonal antibody.

11. (Withdrawn) The method of claim 7 or 8, wherein the TCR-crosslinking agent is a T cell mitogen selected from the group consisting of: concanavalin-A (ConA); phytohemagglutinin (PHA); and pokeweed mitogen (PWM).

12. (Withdrawn) The method of claim 1, 2, or 7, wherein the effective amount of α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof, is an amount sufficient to produce an *in situ* concentration of at least approximately 30 pg/ml of whole α-MSH or an analogue or derivative of α-MSH comprising a molar equivalent amount of an α-MSH receptor-binding portion thereof, in the immediate vicinity of the primed T cells during the exposing step.

13. (Withdrawn) The method of claim 1, 2, or 7, wherein the effective amount of α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof, is an amount sufficient to produce an *in situ* concentration in the range of

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approximately 30-100 pg/ml in the immediate vicinity of the primed T cells during the exposing step.

14. (Withdrawn) The method of claim 3 or 8, wherein the effective amount of TGF-β2 is an amount sufficient to produce an *in situ* TGF-β2 concentration that lies within the range of approximately 1-10 ng/ml in the immediate vicinity of the primed T cells during the exposing step.

15. (Withdrawn) The method of claim 3 or 8, wherein the effective amount of TGF-β2 is an amount sufficient to produce an *in situ* TGF-β2 concentration of approximately 5.0 ng/ml in the immediate vicinity of the primed T cells during the exposing step.

16. (Withdrawn) The method of claim 1, 2, 7 or 8, wherein the exposing step comprises incubating the T cells *in vitro* with the specific antigen and the composition at approximately 37°C, for a period within the range of approximately 18-24 hours, in substantially serum-free T cell culture conditions.

17. (Withdrawn) The method of claim 16, wherein the substantially serum-free T cell medium includes RPMI 1640, an approximately 500-fold dilution of ITS+ solution and approximately 0.1% bovine serum albumin.

18. (Withdrawn) The method of claim 5, 7, 23, 24, or 25, wherein the animal is a human, a mouse, a rat, a dog, a cat, a rabbit, or a horse.

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19. (Withdrawn) A kit for generating antigen-specific regulatory T cells, comprising:

(a) a specific antigen;

(b) α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof; and

(c) an article of manufacture comprising instructions on how to use components (a) and (b) to generate TGF-β-producing, CD4+/CD25+, regulatory T cells.

20. (Withdrawn) The kit of claim 19, further comprising: (d) TGF-β2, and wherein the article of manufacture further comprises instructions for using the TGF-β2.

21. (Withdrawn) The kit of claim 19, wherein the specific antigen comprises a target molecule of an autoimmune disorder.

22. (Withdrawn) The kit of claim 21, wherein the target molecule is selected from the group consisting of: a glycoprotein; a protein; a polypeptide; a synthetic amino acid polypeptide; a recombinant amino acid polypeptide; a carbohydrate moiety; an oligonucleotide; a DNA; a RNA; and a whole microorganism.

23. (Withdrawn) A method for down-regulating a graft rejection response in a graft recipient, comprising:

(a) transfecting a graft tissue or organ with genetic material for expressing α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor binding portion thereof in said graft; and

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(b) implanting the transfected graft from step (a) into a recipient animal.

24. (Original) A method for down-regulating a T cell-mediated autoimmune response in a tissue site in an animal, comprising directly injecting genetic material for expressing α-MSH, into or near the autoimmune-diseased tissue site.

25. (Withdrawn) A method for down-regulating a T-cell-mediated autoimmune response in a tissue site in an animal, comprising:

(a) harvesting a tissue sample from the tissue site;

(b) transfecting the harvested tissue sample with genetic material for expressing α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof; and

(c) implanting the transfected tissue sample into the animal.

26. (Withdrawn) A method of suppressing a T cell-mediated autoimmune graft rejection response in an animal, comprising:

(a) systemically injecting into the animal, an effective amount of α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof; and

(b) measuring the peripheral level of CD4+/CD25+ T cells in said animal.

27. (Withdrawn) The method of claim 26, wherein the effective amount of α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof, is an amount sufficient

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to produce a peripheral blood concentration of at least approximately 30 pg/ml of whole α-MSH or a molar equivalent concentration of an α-MSH receptor-binding portion of α-MSH.

28. (Withdrawn) A method of down-regulating or suppressing an autoimmune disorder or a graft rejection response in an animal by transfecting a cell within the animal with genetic material coding for an antigen that also comprises lysine-proline-valine.

29. (Withdrawn) The method of claim 23, 25, or 28, wherein the transfecting step is performed using an episomal transfection technique.

30. (Withdrawn) The method of claim 23, 25, or 28, wherein the transfecting step is performed using a chromosomal transfection technique.

31. (Withdrawn) A method of regulating a T cell-mediated immune response in a mammal, said method comprising the steps of:

(a) providing a mammal; and

(b) administering to said mammal an effective amount of α-MSH or an analogue or a derivative of α-MSH, said analogue or derivative having α-MSH functional activity, wherein said α-MSH functional activity is mediated exclusively through melanocortin 5 receptor (MC5r),

wherein said step of administering regulates said T cell-mediated immune response.

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32. (Withdrawn) The method of claim 31, wherein said α-MSH is a synthetic analogue wherein said analogue mediates the activation of regulatory T cells.

33. (Withdrawn) The method of claim 31, wherein said α-MSH is attached to a polyclonal or monoclonal antibody, wherein said antibody acts as an agonist to the bound MC5r receptor.

34. (Withdrawn) The method of claim 33 wherein said antibody is an anti-MC5r antibody, or fragment or derivative thereof.

35. (Withdrawn) The method of claim 34 wherein said anti-MC5r antibody is an anti-MC5r antibody $F(ab)_2$ fragment.

36. (Withdrawn) The method of claim 31, wherein said regulation of T cell-mediated immune response is suppression of T cell-mediated inflammatory response.

37. (Withdrawn) The method of claim 31, wherein said regulation of T cell-mediated immune response is induction of $CD4^+/CD25^+$ regulatory T cells that produces TGF-*.

38. (Withdrawn) A method for down-regulating a T cell-mediated autoimmune response in autoimmune disease tissue site in an animal, comprising directly injecting α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof, into or near the autoimmune-diseased tissue site.

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39. (Withdrawn) A method for down-regulating a T-cell-mediated autoimmune response in a tissue site in an animal, comprising:

(a) harvesting a tissue sample from the tissue site;

(b) treating the harvested tissue sample with α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof; and

(c) implanting the treated tissue sample into the animal.

40. (Withdrawn) A method for down-regulating a T cell-mediated autoimmune response in autoimmune disease tissue site in an animal, said method comprising the steps of:

(a) providing said animal; and

(b) directly injecting an effective amount of α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof, into or near the autoimmune-diseased tissue site in an animal; wherein said effective amount is an amount sufficient to produce an *in situ* concentration in the range of about 30-100 pg/ml.

41. (Withdrawn) A method for down-regulating a T-cell-mediated autoimmune response in a tissue site in an animal, comprising:

(a) harvesting a tissue sample from the tissue site;

(b) treating the harvested tissue sample with an effective amount of α-MSH or an analogue or derivative of α-MSH comprising an α-MSH receptor-binding portion thereof; wherein said effective amount is an amount sufficient to produce an *in situ* concentration in the range of about 30-100 pg/ml; and

(c) implanting the treated tissue sample into the animal.

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